Development of novel small molecule EPHB3 inhibitors to treat neurodegenerative disease by targeting astrocyte-mediated disease mechanisms

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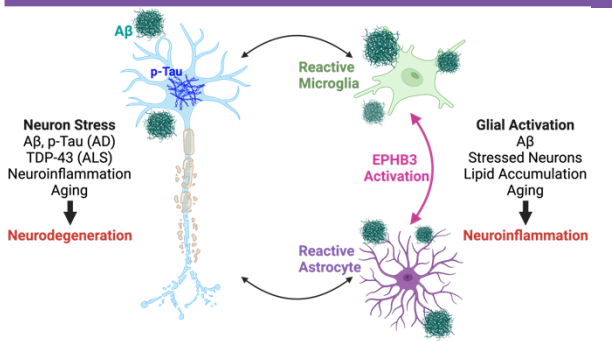
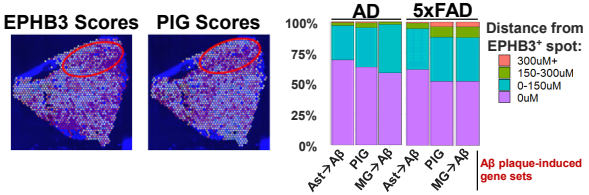
EPHB3 Controls Astrocyte-Microglia Interactions that Drive Neuroinflammation



- EPHB3 is an **astrocyte** (AST) receptor tyrosine kinase
- Binds EFNB3 ligand presented on **microglia** (MG)
- This AST-MG interaction drives neuroinflammation
- Therapeutic Goal = inhibit EPHB3 to protect neurons

EPHB3 Activation is Highly Co-Localized with Aβ Plaque-Induced Gene (PIG) Response in AD



- High co-localization of EPHB3 activation and Aβ plaques in human AD brain and 5xFAD mice by spatial transcriptomics

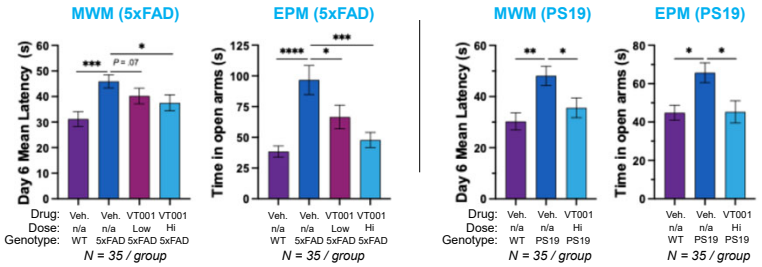
VT-001: a Novel Small Molecule EPHB3 Inhibitor

	Selected assay/ Target	VT-001
Properties	CNS MPO[1]	5
	Kinetic solubility (7.4, uM)	192
	MW, cLogD	<410, <1
	PPB % unbound (m, r, d, c, h)	64, 71, 65, 61, 51
Biochemical	EphB3 (IC50, uM)	0.048
	Carna NanoBRET (196 kinases)	1/240 (EPHB3)
Kpuu	Rat	40%
In vitro tox	CYPs	< 50% @ 10uM
	hERG	37% @ 10uM
	SafetyScreen44	1/44 (3 µM AChE)

VT-001 is:

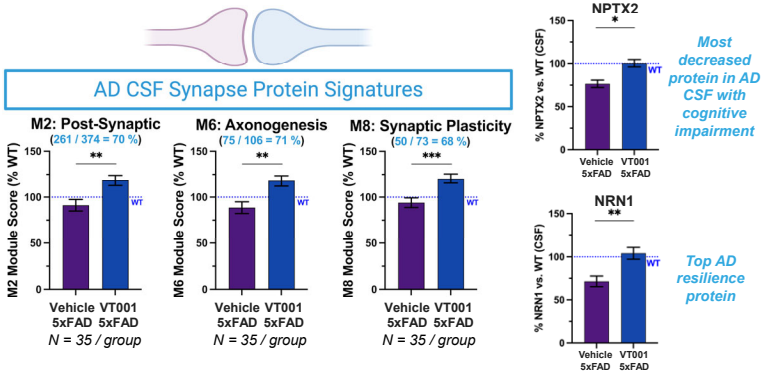
- Very potent
- Highly selective
- CNS-permeable
- Active in acute (LPS) and chronic (EAE) neuroinflammation assays (not shown)

VT-001 is Highly Efficacious in 5xFAD (Aβ) and PS19 (tau) Mouse Models of AD Neuropathology



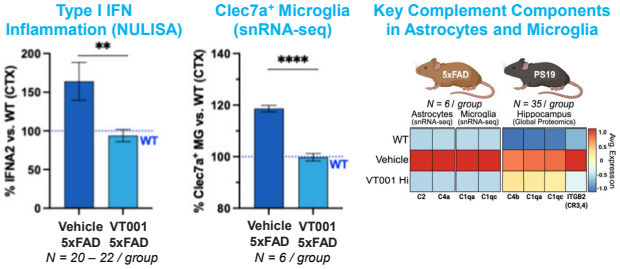
- VT-001 prevents cognitive deficits in 5xFAD mice in the Morris water maze (MWM) and elevated plus maze (EPM) following 2 mos of dosing (6.5 – 8.5 m/o)
- VT-001 prevents cognitive deficits in PS19 mice in the MWM, EPM, social preference test (not shown) following 3 mos of dosing (6 – 9 m/o)
- **Unique efficacy profile:** highly efficacious in amyloidosis and tauopathy models

VT-001 Rescues Proteomic Signatures of AD Synapse Pathology in 5xFAD CSF



- 5xFAD mice show decreased levels of large synaptic protein modules taken from human AD CSF datasets (Bangs, MC, … Seyfried, NT. 2025)
- VT-001 potentiates the levels of these modules significantly above WT
- VT-001 completely rescues the levels of NPTX2 and NRN1, two key AD biomarkers, to WT levels

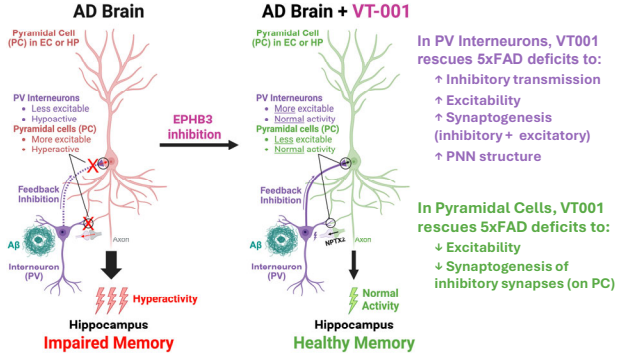
VT-001 Blocks Type-I IFN and Complement Signatures in 5xFAD and PS19 Brain



VT-001 significantly decreases key inflammatory mechanisms in brain known to drive synapse loss in AD:

- Type I IFN protein levels (IFNA2) in 5xFAD neocortex
- Clec7a+ microglia (Type I IFN driver) in 5xFAD neocortex
- Complement components and receptors in 5xFAD and PS19 brain

VT-001 May Restore Memory by Increasing Inhibition in AD Memory Circuits (snRNA-seq)



Ongoing and Future Directions

- Neurohistology in 5xFAD and PS19 to quantify synapse density
- Electophysiology in 5xFAD and PS19 to assess synapse function
- CSF proteomics in PS19 to identify VT-001 biomarkers
- Spatial transcriptomics to establish VT-001 MOA local to Aβ and tau pathology